UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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MEMORANDUM

SUBJECT: **Etofenprox: Response to Company Comments on the Developmental Neurotoxicity Study Review – MRID No. 46062301.**
PC Code: 128965
DP Barcode: 333288; MRID# 46940501
Regulatory Action: Company Response

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THROUGH: Paula Deschamp, Chief
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Action Requested: Respond to company comments on the review of a Developmental Neurotoxicity Study with Etofenprox (MRID# 46062301).

Recommendations: The Agency has reviewed the company comments on the DNT study review and determined that the study remains classified as Acceptable/Nonguideline and recognizes that positive control data was submitted in support of the DNT study; however, HED is in the process of reviewing positive control studies from various laboratories including those conducted at Huntingdon Life Sciences. Many of the developmental neurotoxicity studies submitted to the Agency are currently classified as Acceptable/Nonguideline pending HED's comprehensive review of all submitted positive control data. The classification of the etofenprox DNT study (MRID #46062301) will be reconsidered when HED completes its comprehensive review of all positive control data.

Background: HED received a Company Response to the review of a Developmental Neurotoxicity study with Etofenprox (MRID# 46062301).

Briefly the deficiencies reported in the DER for MRID# 46062301 were:

- Homogeneity and stability analysis were not included in the report.
- Equipment types and descriptions used in the motor activity and the auditory startle tests were not provided in the study report.
- In the memory assessment, there was no evidence for recall/memory. Consequently, additional data are needed to confirm whether different animals were used at different time point and whether intra- or extra maze cues were used in the assessments.

This study is classified **Acceptable/Nonguideline** developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). This study could be considered acceptable if the analytical data for homogeneity and stability analysis are provided. With the analytical data this study may be used for regulatory purposes. This study is considered non-guideline at this time due to deficiency in the memory assessment in the offspring and pending a comprehensive review of all available positive control data.

The company provided a response to the review and this will be discussed as follows:

1. EPA Comment:

Homogeneity and stability analysis were not included in the report. There is a further comment that the concentrations of 100 and 5000 ppm Etofenprox in the diet used for the stability and homogeneity assessments were different to the dietary concentrations used in this study.

Company Response:

The results of the homogeneity and stability analyses are not included in the final report for the MTU/215 study because this work was performed as a separate GLP compliant formulation chemistry study MTU/222. The United Kingdom Good Laboratory Practice Monitoring Authority (UK GLPMA) has ruled that the results from one study may not be presented in detail in the report for another separate study, but may however be briefly referenced. This is the practice that has been followed in the final report for MTU/215 in compliance with UK GLPMA instructions for GLP compliance.

Good homogeneity and stability of Etofenprox in the diets was established at 100 ppm and 5000 ppm on MTU/222. The dietary concentrations of Etofenprox used on MTU/215 were 250 ppm, 700 ppm and 2100 ppm and thus all fell well within the range of concentrations investigated on MTU/222. We are not aware of any GLP or Regulatory requirement that homogeneity and stability have to be assessed at every single concentration used on a programme of work when good data is available for higher and

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lower concentrations; the practice we have used of assessing the highest and lowest concentrations predicted for forthcoming toxicology studies is our standard practice and is acceptable to the GLP and regulatory authorities. We consider the concentrations at which stability and homogeneity were investigated on MTU/222 adequate to support the concentrations used on MTU/215.

The stand alone homogeneity and stability study (MTUl222) is submitted with these comments.

EPA Response:

The provided report, MTU/222 with EPA MRID# 46940501, is a stand alone document of the analysis of Etofenprox in the UAR VRF1 Certified Diet (VRF1). This report was intended to be used for support of dietary administration studies with Etofenprox. The investigators looked at a dietary admix of 100 and 5000 ppm both with and without corn oil ("plain") in the admix.

From the investigators report:

Response was found to be linear with respect to concentration, independent of order of injection and reproducible. Injection reproducibility for a standard solution and for an extract of Etofenprox in VRF1 diet at approximately 100 ppm were found to be acceptable.

Two different batches of plain VRF1 diet and one batch of VRFl diet incorporating corn oil were analysed. They were found to produce insignificant matrix interference at the characteristic retention time of Etofenprox at the dilutions required for analysis. The accuracy and precision of fortifications prepared at the highest (5000 ppm) and lowest (100 ppm) concentrations predicted for forthcoming toxicology studies were evaluated on two separate occasions. The analytical method was found to be precise and accurate at both concentrations. The inclusion of corn oil in the diet was found to have no significant impact on the chromatography, accuracy or precision.

The limit of quantitation for Etofenprox in VRFl diet demonstrated in this study was 100 ppm. the lowest concentration of Etofenprox used. The limit of detection was 4 ppm.

Trial formulations of Etofenprox were prepared in VRFl diet at the highest (5000 ppm) and lowest (100 ppm) concentrations predicted for forthcoming toxicology studies. The trial formulations were assessed for homogeneity of mixing using the validated analytical method. Stability of the trial preparations at nominal room temperature was also assessed in order to determine the frequency of preparation of diet formulations for the forthcoming toxicology studies. The trial formulations were both found to be homogenous. The nominal 5000 ppm formulation was found to be stable for at least 22 days. The nominal 100 ppm formulation was found to be stable for 15 days but unsatisfactory after 22 days storage.

The above statements are supported by the submitted data. The test procedures were accurate and acceptable for determining the concentration, homogeneity and storage stability of the test compound in the diet. The concentration analysis showed nearly 100% of the target concentration. The homogeneity was within acceptable limits. The storage stability of the test compound-dietary admixes were satisfactory at 8 and 15 days for the 100 ppm dietary admixes with a loss of less than 5%; however, this dropped to

nearly 10% at 22 days. The 5000 ppm dietary admix was stable at both 8 and 22 days with a loss of approximately 6% by day 22.

This adequately addresses the deficiency "Homogeneity and stability analysis were not included in the report", completely.

2. EPA Comment:

Equipment types and descriptions used in the motor activity and the auditory startle tests were not provided in the study report.

Company Response:

It is always difficult to know exactly how much detail to include in a study report: this is not a comment that we have previously received from US EPA following review of DNT study reports.

The following additional information is provided and can be used to support the MTU/215 study.

The motor activity equipment is described in detail on page 30 of the report. The equipment was made by Pearson Technical Services Framlingham, Suffolk, England. The software was written and is supported by the IT Department of HLS. Each animal station operates independently, starting its one hour of counting from the initial beam breaks when the animal is placed in the cage. The test cage size was approximately 54 cm long x 34 cm wide x 19 cm high. Each station is visually isolated by white Perspex sheeting so animals in adjacent stations cannot be seen. The animals are left in a room undisturbed during the test period of one hour. Animals are, as far as possible, evenly distributed by group and sex between racks and stations.

The auditory startle equipment used was the Startle Responder-X, a well-known system available from Columbus Instruments. The system consists of four sound attenuating boxes, each containing a loudspeaker and a force platform within a Perspex chamber. These are all connected to a central control unit and PC. The experimental details (noise levels and trial types etc.) for startle habituation and pre-pulse inhibition are described in full on page 31 of the report.

EPA Response:

The guidance for what is needed in the study report is provided in the guideline OPPTS 870.6300. The above provided information is adequate to address the study deficiency "Equipment types and descriptions used in the motor activity and the auditory startle tests were not provided in the study report."

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3. **EPA Comment:**

In the memory assessment, there was no evidence for recall/memory. Consequently, additional data are needed to confirm whether different animals were used at different time points and whether intra-or extra maze cues were used in the assessments.

Company Response:

The results of the Morris water maze testing show clear decreases in mean trial times and pool sector entries and the mean number of failed trials over the four days of testing. The progressive improvement in the performance of the animals is clear evidence of learning (acquisition) and subsequent recall/memory from day to day.

The actual regulatory requirement detailed in the US EPA OPPTS 870.6300 guideline is for the test to "include some measure of memory (short-term or long-term) in addition to original learning (acquisition)." We believe that the improvement in performance from Day 1 to Day 2 of testing reflects short-term memory on Day 1 being consolidated into acquisition learning evident on Day 2, while the overall improvement in performance over the 4 days of testing satisfies the requirement for a measure of long-term memory.

"While there is no absolute agreement as to where the line is drawn between short-term and long-term memory, it appears that the ability to remember for more than a day is evidence of long-term memory (Quoted by US Dept of Health and Human Services NIH News. 14 October 2004). Indeed the cut-off at one day between short and long term memory is longer than most sources we have seen where it tends to be that anything, more than 30 seconds or a few minutes is viewed as long term memory".

It was not possible to demonstrate memory over a long time period on the study because a different set of offspring was tested at about Day 60 of age than that tested shortly after weaning. The EPA reviewed the proposed protocol for the study and sanctioned this design. In a letter dated July 31 2002 concerning the proposed assessment of learning and memory in different sets of offspring shortly after Day 21 of age and around Day 60 of age the EPA stated that "In general, the use of separate animals at the two time points is preferred because for many tasks initial learning may confound later (PND 60) assessment of learning. The submitted protocol specifies the use of separate animals for assessment at the two time points."

The materials and methods section of the report (page 27) details that one set of offspring were tested in the water maze at day 23/24 of age (set 4) with a different group of offspring (set 5) tested in the maze at day 58/59. The identities of individual animals tested on each occasion are detailed in appendices 38 and 39 and this confirms that different sets of animals were tested at day 23/24 and days 58/59.

The materials and method section of the report (page 31) explains that both intra- and extra maze cues were used in the assessments.

EPA Response:

The above information provided adequately addresses the Agency concerns on the deficiency "In the memory assessment, there was no evidence for recall/memory. Consequently, additional data are needed to confirm whether different animals were used at different time point and whether intra- or extra maze cues were used in the assessments."

4. EPA Comment:

There is also a comment at the end of the study deficiencies section that the study is considered non-guideline at this time due to deficiency in the memory assessment in the offspring and pending a comprehensive review of all available positive control data.

Company Response:

Positive control data submitted to US EPA to date in support of developmental neurotoxicity studies Huntingdon Life Sciences are as follows:

Automated motor activity monitoring – offspring

Study HLS/058: Assessments of the effects of amphetamine or chlorpromazine on the motor activity of young rats - positive control study, and examination of brains from untreated 11-day old rats; MRID 45308301.

Morris water maze and auditory startle response

Study HLS/059: Assessment of the effect of scopolamine on auditory startle response and Morris water maze learning in young rats. Positive control study; MRID 45308302.

Study HLS/248: Assessment of the effect of scopolamine on Morris water maze learning, and assessment of auditory startle response habituation in young rats. MRID 46484601

Study HLS/275: Validation of the columbus responder-X automated startle response system and investigation into optimal experimental conditions in the CD rat. MRID 46483602

HLS/317: Further validation of Morris water maze procedures in young rats using Scopolamine. *Final Report submitted March 2006, MRID number unknown.*

Functional observational battery – offspring

Study HLS/088: Validation of functional observational battery for pre-weaning rat pups in developmental neurotoxicity screening. MRID 46483603

Functional observational battery - adult animals

HLS/027 (Report No. 98 2493). Further Validation Of Neurotoxicity Procedures Following Oral Gavage Administration Of D-Amphetamine Or Diisoprpyl Fluorophosphate To CD Rats To Meet EPA FIFRA Requirements. Final Report Issued: 14 January 2002. Author: N Bolton. MRID No. 46474222

LSRIO30 (Report No. 93/LSR030/0912). MRID 43680415.

LSRJ031 (Report No. 94/LSR031/1172). MRID 43680114.

Neuropathology- adult animals

R&D 91A/01A(4)/942639 - reports effects of acrylamide - axonal degeneration in peripheral nerves. MRID 41117801.

R&D 91A/01C(2)/942873 - reports effects of trimethyltin chloride - loss of neurons and gliosis in hippocampus; pyriform cortex also affected in males. MRID 14447802.

R&D 91A/01C(1)/942872 - Trimethyltin Chloride (TMT): Pilot Toxicity Study- In Rats For Neurotoxicity Testing. Final Report Issued: 10 June 1996. Author: EW Hughes.

Reports effects of trimethyltin chloride - loss of neurones/neuronal necrosis/gliosis in the hippocampus and in the pyriform cortex. Also Gitter cells /gliosis/axonal degeneration in the midbrain/cerebellum/pons/medulla.

EPA Response:

The Agency recognizes the fact that these positive control studies and reviews were submitted; however, HED is in the process of reviewing positive control studies from various laboratories including those conducted at Huntingdon Life Sciences. Many of the developmental neurotoxicity studies submitted to the Agency are currently classified as Acceptable/Nonguideline pending HED's comprehensive review of all submitted positive control data. The classification of the etofenprox DNT study (MRID #46062301) will be reconsidered when HED completes its comprehensive review of all positive control data.

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Chemical: Ethofenprox

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